



Evaluation of incorporation and optimization of different types of propolis on the physicochemical properties of natural rubber (*Hevea brasiliensis*) membranes

Evelin Thayná Barbosa Serpa^{1,2} · Bruna V. Quevedo^{2,3} ·
Eliana Aparecida de Rezende Duek^{2,3} · Maira de Lourdes Rezende^{1,2}

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Abstract

The incorporation of propolis into natural rubber (NR) latex has attracted increasing scientific interest, as it combines the biocompatibility of NR with the therapeutic properties of propolis. However, the most suitable form of propolis (aqueous, ethanolic, or dry) to optimize its interaction with the polymeric matrix and its bioactive performance remains uncertain. In this study, the physicochemical properties of NR membranes incorporated with different forms of propolis - dry extract (PDE), aqueous extract (AEP), and ethanolic extract (EEP) - at concentrations of 5%, 10%, and 20% were investigated. Fourier-transform infrared spectroscopy (FTIR) confirmed physical interactions between the constituents, promoting the controlled release (140 h) of active compounds. Kinetic analysis fitted the Korsmeyer-Peppas model ($n < 0.5$), indicating a Fickian diffusion mechanism. Scanning electron microscopy (SEM) revealed that the incorporation of propolis increased the surface roughness of the NR membrane. Thermogravimetric analysis (TGA) showed that the addition of propolis did not compromise the thermal stability of NR (~ 360 °C). Differential scanning calorimetry (DSC) revealed that PDE and EEP did not affect the glass transition temperature ($T_g = -65$ °C) of NR, whereas AEP induced a shift to approximately -3 °C, reducing the polymer's flexibility. Contact angle measurements indicated that all membranes remained hydrophilic (angles $< 90^\circ$). Swelling assays further indicated that the presence of propolis significantly influenced the fluid absorption capacity of the membranes. The results indicate that propolis type and concentration influence NR's physicochemical properties and release behavior, offering insights for developing bioactive and regenerative biomaterials.

Keywords Propolis extract · *Hevea brasiliensis* · Biomaterial · Biopolymer · Sustainability · Biomedical application

Introduction

Polymeric biomaterials have received considerable attention in the biomedical field due to their versatility, enabling applications in tissue engineering and controlled drug delivery systems [1]. These materials are classified as synthetic or natural, each with distinct advantages and limitations. Synthetic polymers precise control over mechanical properties, composition, and reproducibility, whereas natural polymers offer high biocompatibility, biodegradability, and structural similarity to the extracellular matrix, promoting cell adhesion, proliferation, and differentiation while mimicking intrinsic biological processes [1, 2]. These characteristics support the scientific interest in natural polymers for developing innovative therapeutic strategies.

Among natural polymers, natural rubber latex (NR), obtained from *Hevea brasiliensis*, has emerged as a sustainable and versatile biomaterial. Chemically, NR is primarily composed of macromolecule poly(cis-1,4-isoprene). Its colloidal suspension contains approximately 50% water, 40–45% rubber, and 4–5% proteins and lipids [3]. NR has demonstrated multiple therapeutic properties, including the promotion of tissue repair [3, 4], stimulation of angiogenesis [5], and enhancement of cell adhesion and proliferation [6]. Additionally, NR has shown potential as a matrix for controlled drug delivery [7, 8]. These features make NR particularly suitable for applications in dermal dressings and bioactive systems, which can be further optimized through the incorporation of therapeutic agents.

In this context, propolis is recognized as a bioactive compound of significant interest. It is a natural resin produced by bees from plant exudates, combined with wax and enzymes, and serves as a protective barrier against microorganisms within the hive [9]. Propolis has a highly complex composition, primarily consisting of plant resins, with smaller fractions of waxes, essential and aromatic oils, pollen, and other organic compounds [10]. To date, over 300 chemical constituents have been identified, with phenolic compounds, flavonoids, terpenes, and other bioactive metabolites predominating. These components are largely responsible for propolis' well-documented antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties [10–12]. Brazil harbors one of the greatest diversities of propolis worldwide, encompassing 13 distinct types whose variations are directly associated with their botanical origin, such as the green propolis from *Baccharis dracunculifolia*, red propolis from *Dalbergia ecastaphyllum*, and brown propolis from *Hyptis divaricata* [10, 12]. This chemical diversity results in differentiation underlies the varied biological activities of propolis, supporting its widespread use in pharmaceutical and biomedical applications, including capsules, sprays, lotions, and ointments [13].

Recent studies have reinforced the therapeutic potential of propolis across diverse medical applications. Hossain et al. [14] and Pereira et al. [15] highlighted its effectiveness in treating wounds and ulcers. Variani et al. [16] evaluated propolis ointment for the herpetic lesions, while Munhoz et al. [17] reported promising wound-healing effects of a propolis-based cream in burn injuries models. Tiang et al. [18] highlighted its diabetes management, improving glycemic control, lipid metabolism, and liver function. Additionally, Shakeri et al. [19] developed a bifunctional multilayer nanofibrous mucoadhesive patch incorporating propolis for sustained drug release via electrospinning. This system demonstrates potential as a localized, controlled

delivery platform, leveraging the anti-inflammatory and antimicrobial properties of propolis for oral mucosal therapies.

The combination of NR and propolis for biomedical applications has been extensively studied. Silva et al. [20] demonstrated that NR membranes incorporated with propolis effectively inhibit *Candida albicans* growth and reduce pain during dressing changes, highlighting their potential for burn treatment. Zancanela et al. [21] investigated different propolis types (red, green, and álamo) in NR matrices, reporting controlled antimicrobial release, enhanced wound healing, and inhibition of *C. albicans*. Krupp et al. [22] observed that NR membranes containing *Scaptotrigona polysticta* propolis accelerated tissue repair in second-degree burns. Garcia et al. [23] developed NR films enriched with red propolis and silver nanoparticles, achieving bactericidal activity and high hydrophobicity suitable for biomedical applications. More recently, Bystronski et al. [24] proposed NR films combining red propolis with copper, offering a promising alternative to conventional wound dressings.

Despite advances in the incorporation of bioactive compounds into NR matrices, including propolis, a significant gap remains regarding the identification of the most suitable propolis form - aqueous, ethanolic, or dry - to optimize its interaction with the polymeric matrix. This selection is crucial, as different propolis forms vary in solubility, stability, and chemical composition, which directly affect incorporation efficiency, release behavior, and biological performance. Given the established potential of NR as a biomaterial and the wide range of therapeutic properties attributed to propolis, this study systematically compares different propolis forms incorporated into NR to determine the formulation that offers superior physicochemical characteristics and bioactive potential. The findings aim to support the rational design of NR-based biomaterials for wound dressings and regenerative medical applications.

Materials and methods

Materials

Natural rubber latex (NR), ammonia-stabilized (pH ~ 10), was supplied by São Roque Latex Artifacts Factory Ltd. (Brazil). Hydrochloric acid (0.5 mol/L) was obtained from Anidrol (Brazil). Chloroform (CHCl₃, 99.8%) and methanol (99.8%) were purchased from Synth (Brazil). Green propolis dry extract (PDE), produced by spray drying with water as solvent, was acquired from Henrifarma Produtos Químicos e Fármacos LTDA (Brazil). Aqueous extract of propolis (AEP, 11% w/v in deionized water) and the ethanolic extract of propolis (EEP) (11% w/v in neutral alcohol) were obtained from Ecoas (Brazil). All propolis extracts were used as received, without further purification.

Preparation of deproteinized natural rubber

NR was deproteinized by solvent reprecipitation following the method reported by Quevedo et al. [25]. Initially, latex was neutralized by the addition of 2.0 M hydrochloric acid until pH 7.2, inducing precipitation. The precipitated NR was solubi-

lized in chloroform and subsequently reprecipitated in methanol. This process was repeated to enhance protein removal. The purified material was dried at room temperature for 72 h and stored under vacuum desiccator.

Preparation of membranes from deproteinized natural rubber loaded with different propolis extracts

NR was dissolved in chloroform (10% w/v). PDE was incorporated at 5%, 10%, and 20% (w/w) relative to the polymers mass, and the mixture was stirred for 24 h to ensure homogeneous dispersion. Using the same procedure, AEP and EEP were added to the NR solution at concentrations of 5%, 10%, and 20% (v/w). The resulting formulations were cast onto Teflon plates and dried at room temperature for 48 h to form membranes.

Characterization

Fourier transform infrared spectroscopy (FTIR–ATR)

The FTIR analysis was conducted in an ATR module on a Spectrum 65 model spectrophotometer (Perkin Elmer, USA), with a wavenumber in the range of 4000 to 500 cm^{-1} , employing a resolution of 4 cm^{-1} and 32 scans.

Scanning electron microscopy (SEM)

The surface morphology of the membranes (NR and NR incorporated with different types of propolis (NR+PDE, NR+AEP, and NR+EEP) at concentrations of 5%, 10%, and 20%) was analyzed using scanning electron microscopy (SEM; Hitachi TM3000) at an accelerating voltage of 5 kV.

Thermal analysis

Thermogravimetry analysis (TGA) was performed using Discovery TGA 55 equipment (TA Instruments, USA) over a temperature range of 25–500 °C. Measurements were conducted under a dynamic nitrogen atmosphere at a flow rate of 40 mL/min with heating rate of 10 °C/min. Differential Scanning Calorimetry (DSC) was performed using Discovery DSC 25 equipment (TA Instruments, USA) under a nitrogen atmosphere at 250 mL/min. Propolis samples (PDE, AEP, and EEP) were heated from –70 °C to 220 °C. Membrane samples composed of NR incorporated with different types of propolis (NR+PDE, NR+AEP, and NR+EEP) at concentrations of 5%, 10%, and 20% were subjected to a multi-step thermal program. This program consisted of heating from 25 °C to 120 °C, followed by a 1-min isothermal hold, subsequent cooling to –85 °C with a 1-min isothermal hold, and a second heating step to 220 °C. All steps were conducted at a heating/cooling rate of 10 °C/min.

Contact angle measurement

Measurements of contact angles with deionized water were performed in triplicate using a goniometer (Ramé-hart Instrument Co., USA) and DROPimage Standard software. Approximately 20 μL of deionized water (a polar liquid) was carefully deposited on the membrane's surface using the software-controlled equipment. Contact angle values were determined from these images, and the result was obtained as the average of ten measurements.

Swelling test

The samples (~ 0.5 g) were weighed and placed in a Falcon tube containing 10 mL of 0.01 M phosphate-buffered saline solution (PBS) and maintained in a thermostatic bath at a temperature of 36 °C. Measurements were conducted over a predetermined period until completing a total of 168 h of analysis. For weighing, the samples were removed from the Falcon tube and dried with paper towels to remove excess water from their surface. Swelling tests were performed in triplicate. The percentage of volume increment (% swelling) for each sample was calculated from its initial weight (W_0) and its weight after absorption (W_t), as determined by Eq. 1.

$$\text{Swelling (\%)} = (W_0 - W_t/W_0) \times 100 \quad (1)$$

In vitro release profile

The in vitro release profile was performed in triplicate using samples (with an area of 1 cm^2) of NR membranes incorporated with 5%, 10%, and 20% of different types of propolis (PDE, AEP, and EEP). The samples were immersed in Falcon tubes containing 6 mL of 0.01 M phosphate-buffered saline (PBS) and kept at 37 °C in a thermostatic bath. Then, 3 mL aliquots were collected at predetermined time intervals until 140 h of testing were completed. The volume removed in each aliquot was replaced with PBS solution after each collection. The aliquots were analyzed using a spectrophotometer (Femto model 800 XI) at a wavelength of 250 and 330 nm. Additionally, a calibration curve was constructed using known concentrations of propolis extract as the standard. The calibration curve showed a linear relationship between absorbance and concentration, with the regression equation $y = 18.256x + 0.3984$ and a correlation coefficient of $R^2 = 0.9982$.

Study release mechanism The release mechanism of the different propolis groups incorporated into NR membranes was analyzed by fitting the data to the Korsmeyer-

Peppas (Eq. 2), Higuchi (Eq. 3), Zero Order (Eq. 4), and First Order (Eq. 5) mathematical models [26].

$$\text{Korsmeyer-Peppas} - M_t/M_\infty = k_{kp}t^n \quad (2)$$

$$\text{Higuchi} - M_t/M_\infty = k_H.t^{1/2} \quad (3)$$

$$\text{Zero Order} - M_t/M_\infty = k_0t \quad (4)$$

$$\text{First Order} - M_t/M_\infty = 1 - e^{?k_1t} \quad (5)$$

Where M_t is the amount of propolis release and, t , k_H , k , and K_0 are constant.

Statistical analysis

The results obtained for each group (NR+PDE, NR+AEP, and NR+EEP) are presented as mean $\pm$ standard deviation (SD). Differences between groups were evaluated using one-way ANOVA followed by Tukey's post hoc test. Statistical analysis was performed using Origin software, with significance levels set at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

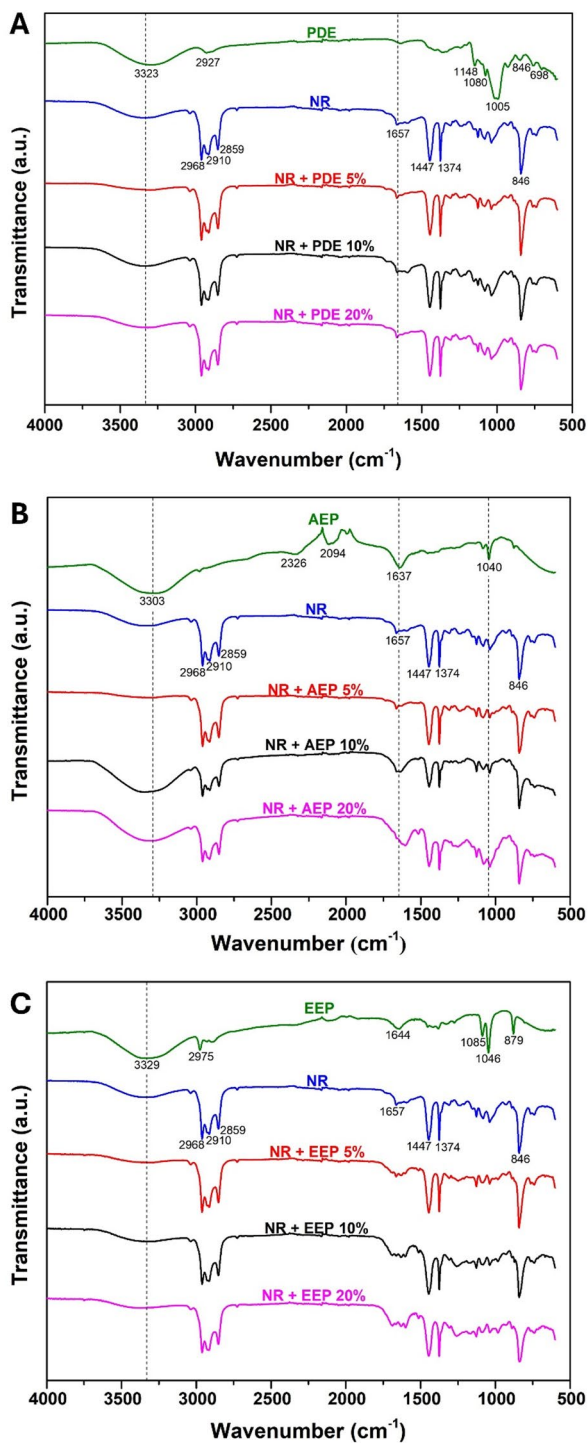
Results and discussion

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the samples, including neat NR and NR incorporated with different concentrations (5%, 10%, and 20%) of propolis extracts (PDE, AEP, and EEP), are presented in Fig. 1. As expected, the spectrum of NR exhibited the characteristic absorption peaks of polyisoprene. The peak at 2968 cm^{-1} was attributed to the asymmetric stretching of $-\text{CH}_3$, while the peaks at 2910 and 2859 cm^{-1} correspond to the symmetric stretching of $-\text{CH}_2$ groups. The absorption at 1657 cm^{-1} was associated with $\text{C}=\text{C}$ stretching vibration. The peaks observed at 1447 cm^{-1} and 1374 cm^{-1} were assigned to the angular deformations of CH_2 and CH_3 , respectively. Finally, the peak at 846 cm^{-1} was attributed to the out-of-plane of $=\text{CH}$ bending in cis-1,4-polyisoprene [27, 28].

The FTIR spectrum of the PDE (Fig. 1A) exhibited a broad peak at 3323 cm^{-1} , attributed to hydrogen-bonded $-\text{OH}$ and N-H groups in polyphenolic compounds, carbohydrates, proteins, or residual water [29]. The peak at 2927 cm^{-1} was assigned to C-H stretching. Peaks at 1148 , 1080 , and 1005 cm^{-1} were attributed to $-\text{C-OH}$ and

Fig. 1 FTIR spectra. The graph presents spectra of natural rubber (NR) and NR incorporated with different concentrations of propolis (5%, 10%, and 20%): **(A)** propolis dry extract (PDE) **(B)** aqueous extract of propolis (AEP), and **(C)** ethanolic extract of propolis (EEP)



-Ar-OH vibrations [30]. Additional peaks observed at 846 and 698 cm^{-1} were associated to C-H bending vibrations of aromatic rings present in phenolic compounds [29, 31]. In contrast, the AEP spectrum (Fig. 1B) displayed distinct features. A broad peak at 3303 cm^{-1} was associated to -OH groups from hydroxyl molecules. Peaks at 2326 and 2094 cm^{-1} were assigned to C-H stretching vibration, while the peak at 1637 cm^{-1} corresponded to C = O stretching of asymmetric flavonoids. The peak at 1040 cm^{-1} was attributed to angular deformation of C = C [32, 33]. The FTIR spectrum of EEP (Fig. 1C) exhibited a broad peak at 3329 cm^{-1} , associated with O-H stretching of phenolic compounds. The peak at 2975 cm^{-1} corresponded to asymmetric CH_2 stretching of ethanol. The peak at 1644 cm^{-1} was assigned to C = C stretching in aromatic rings. Peaks at 1085 and 1046 cm^{-1} were associated with C-O stretching in flavonoids and terpenes and C-OH bending from alcohol and ether, respectively. The peak at 879 cm^{-1} was attributed to functional groups of primary and secondary alcohols [34–36].

The differences observed in the FTIR spectra of the various types of propolis (PDE, AEP, and EEP) reflect variations in their phytochemical composition. The identified flavonoids may exhibit anti-inflammatory, antioxidant, and antiviral activities, as well as promote angiogenesis and tissue regeneration. Terpenes show antimicrobial activity and may enhance wound healing, while phenolic compounds stimulate collagen production in fibroblasts, contributing to tissue repair [37].

In the spectra of NR incorporated with the different types of propolis, the main characteristic peaks of NR were preserved. For PDE (Fig. 1A) and EEP (Fig. 1C), no significant changes were observed, except for an increased intensity of the peak at 3323 cm^{-1} for NR+PDE 10% and NR+PDE 20%, indicating the presence of the extract. Additionally, a slight reduction in the intensity of the peak at 1657 cm^{-1} was observed for the NR+PDE 5% and NR+PDE 20% samples compared to NR+PDE 10%, which exhibited a spectral profile similar to that of pure NR in this region. This behavior may be attributed to possible variations in the dispersion of PDE within the NR matrix.

Furthermore, the main characteristic peaks of NR may overlap with those of the propolis extracts. In NR incorporated with AEP (Fig. 1B), the spectra showed a combination of the peaks of both NR and AEP, with intensified signals at 3303, 1637 and 1040 cm^{-1} , confirming the successful incorporation of AEP. These results suggest that the incorporation of propolis into the NR matrix occurs predominantly through physical interactions between the components, with no evidence of significant chemical modifications [38]. Such physical interactions may facilitate the release of propolis compounds in the wound environment [23].

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was employed to evaluate the surface morphology of pure NR membranes and NR formulations incorporated with different concentrations (5%, 10%, and 20%) of propolis extracts (PDE, AEP, and EEP), as shown in Fig. 2. The micrograph of NR revealed a homogeneous and smooth surface, without the presence of pores, a typical characteristic of this elastomer, as reported in the literature [6, 8, 39]. This morphology may be associated with the deproteinization

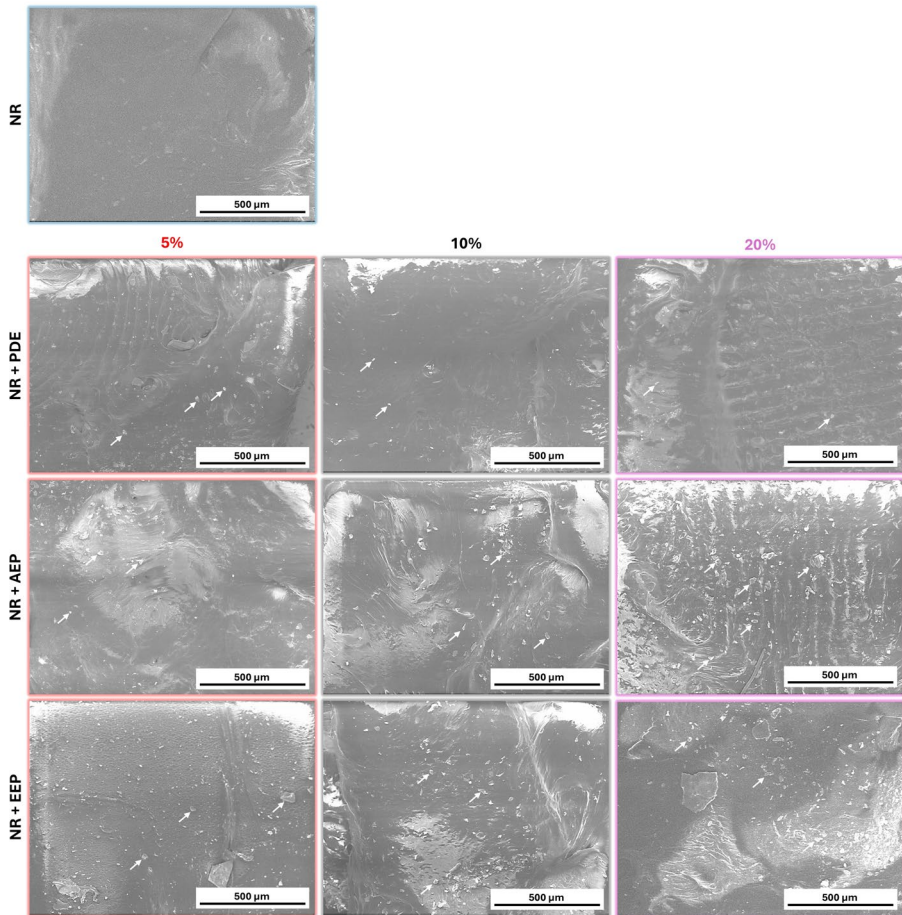


Fig. 2 SEM images. Micrographs of the surface of natural rubber (NR) and NR incorporated with different concentrations (5%, 10%, and 20%) of propolis dry extract (NR+PDE), aqueous extract of propolis (NR+AEP), and ethanolic extract of propolis (NR+EEP). The arrows indicate the presence of propolis in NR. (Scale bar: 500 μm; Magnification: 120x)

process, which promotes the removal of non-elastomeric impurities, such as proteins and lipids, resulting in a more uniform matrix with a reduced presence of surface irregularities [6].

The incorporation of distinct propolis extracts (PDE, AEP, and EEP) into the NR matrix induced morphology changes dependent on both the type and concentration of the extract. Formulations containing 5% and 10% propolis extract exhibited predominantly smooth surfaces with discreetly heterogeneous regions. In contrast, the incorporation of 20% extract resulted in more irregular and textured surfaces, with localized undulations and discontinuities, being particularly pronounced in NR + PDE and NR + AEP. This behavior may be associated with greater interference of the extract constituents in the organization of the elastomeric matrix, as well as the effects of the membrane drying process [6].

Furthermore, all propolis-containing formulations (NR + PDE, NR + AEP, and NR + EEP) exhibited small particles dispersed across the NR matrix surface (indicated by arrows). These deposits are likely attributable to the presence of resinous particles from the propolis extracts that remained after the drying process, indicating that some extract constituents were not fully incorporated into the polymer matrix and were instead locally distributed on the surface. Similar observations have been reported for NR membranes incorporated with propolis [40], NR with zein nanoparticles containing propolis in methylcellulose membranes [41], NR with amphotericin B [39], and NR incorporated with ZnO [8]. Conversely, the formation of rougher surfaces observed in the propolis-containing samples may be advantageous for biomedical applications, such as wound healing, as it promotes improved cell adhesion and proliferation [42].

Thermal analysis

The thermal stability of the different propolis extracts (PDE, AEP, and EEP), NR, and NR incorporated with distinct propolis concentrations (5%, 10%, and 20%) was evaluated by thermogravimetric analysis and its derivative (TGA/DTG; Fig. 3A–C). Thermal transitions were further investigated by differential scanning calorimetry (DSC; Fig. 3D–F). The main parameters obtained from the TGA/DTG analyses are summarized in Table 1.

The TGA of propolis-free NR (Fig. 3A–C) revealed a single and pronounced mass loss event ($\sim 99\%$). The onset temperature of mass loss (T_{onset}) was observed at 340 °C, while the temperature at which the maximum mass loss rate occurs (T_{max}), determined from the DTG curve, was recorded at 360 °C. This behavior is characteristic of the thermal degradation process of NR, predominantly associated with the scission of polyisoprene chains [6, 43].

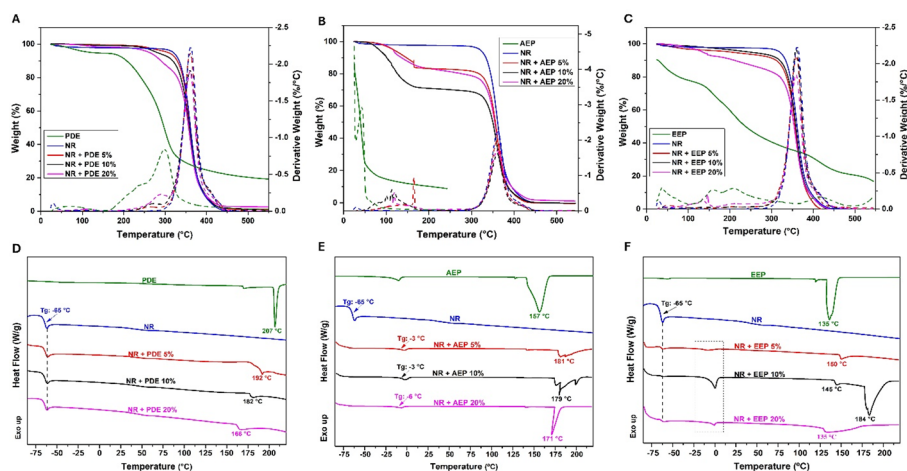


Fig. 3 Thermal analysis of natural rubber (NR) and NR incorporated with propolis extracts at 5%, 10%, and 20%. TGA/DTG and DSC curves are shown for membranes containing (A, D) propolis dry extract (PDE), (B, E) aqueous extract of propolis (AEP), and (C, F) ethanolic extract of propolis (EEP)

Table 1 Thermal properties obtained from TGA and DTG curves of NR, PDE, AEP, EEP, and NR + PDE, NR + AEP, and NR + EEP incorporated with different concentrations of propolis (5%, 10%, and 20%)

Sample	Events (n°)	T_{onset} ($^\circ\text{C}$)	T_{max} ($^\circ\text{C}$)	Weight Loss (%)
NR	1	340	360	99
PDE	1	30	72	5
	2	245	298	70
NR + PDE 5%	1	337	360	99
NR + PDE 10%	1	335	360	99
NR + PDE 20%	1	256	290	10
	2	335	360	85
AEP	1	25	43	80
NR + AEP 5%	1	94	165	17
	2	339	361	83
NR + AEP 10%	1	98	112	29
	2	337	360	71
NR + AEP 20%	1	112	118	17
	2	337	360	81
EEP	1	31	36	12
	2	139	160/207	43
	3	411	415	17
NR + EEP 5%	1	321	360	99
NR + EEP 10%	1	338	360	99
NR + EEP 20%	1	80	135	7
	2	338	358	93

The TGA curve of PDE (Fig. 3A) exhibited an initial mass loss of approximately 5% at temperatures below 100 $^\circ\text{C}$, attributed to the evaporation of residual volatile compounds, such as adsorbed water and small organic molecules present in propolis. With increasing temperature, a main thermal degradation stage was observed, characterized by a mass loss of approximately 70%, with a T_{onset} at 245 $^\circ\text{C}$ and a T_{max} at 298 $^\circ\text{C}$. This stage is associated with the decomposition of the main bioactive constituents of propolis, such as flavonoids and phenolic compounds, which are widely recognized for their relevance in therapeutic and biological applications [30, 44]. The incorporation of 5% and 10% PDE into NR (Fig. 3A) resulted in a single predominant mass loss event ($\sim 99\%$), with a T_{onset} of approximately 337 $^\circ\text{C}$ and a T_{max} of 360 $^\circ\text{C}$, similar to the thermal behavior observed for NR without PDE addition. In contrast, NR + PDE 20% exhibited two distinct thermal events. The first event, with a T_{onset} at 256 $^\circ\text{C}$ and a T_{max} at 290 $^\circ\text{C}$, corresponding to approximately 10% mass loss, was attributed to the degradation of PDE present at higher concentration within the NR matrix. The second event, responsible for 85% mass loss, occurred at a T_{onset} of 335 $^\circ\text{C}$ and a T_{max} of 360 $^\circ\text{C}$, and was also associated with the degradation of NR [45, 46].

AEP (Fig. 3B) exhibited a single pronounced mass loss event, corresponding to approximately 80%, with a T_{max} recorded at 43 $^\circ\text{C}$. This behavior can be attributed to sample volatilization, since the analysis was performed with the material in its liquid state, thereby promoting the evaporation of its volatile components. In turn, all NR + AEP samples (Fig. 3B) displayed two mass loss events. The first, with a T_{onset} of approximately 100 $^\circ\text{C}$, was associated with the degradation of AEP constituents,

particularly flavonoids [46], whereas the second ($T_{\text{onset}} \sim 337^\circ\text{C}$ and $T_{\text{max}} \sim 360^\circ\text{C}$) was also attributed to the degradation of NR [45, 46].

In contrast, EEP (Fig. 3C) exhibited three consecutive mass loss events. The first event, observed at temperatures below 100°C , was attributed to the volatilization of residual water, traces of solvent (ethanol), and other volatile compounds. The second event showed a T_{onset} at 139°C and two T_{max} values at 160°C and 207°C , indicating the decomposition of different organic fractions present in the extract. The third event occurred with a T_{onset} at 411°C and a T_{max} at 415°C . These thermal events were associated with the gradual degradation of the bioactive constituents of EEP. Simpler and more volatile molecules tend to decompose at lower temperatures due to their higher thermal sensitivity. In contrast, more complex aromatic compounds exhibit greater thermal stability, typically degrading at temperatures around 380°C [47, 48]. Like the behavior observed for NR + PDE (Fig. 3A), the NR + EEP 5% and NR + EEP 10% samples (Fig. 3C) exhibited a single main mass loss event, with a T_{onset} of approximately 338°C and a T_{max} at 360°C . In contrast, NR + EEP 20% displayed two distinct mass loss events. The first, with a T_{onset} at 80°C and a T_{max} at 135°C , was attributed to the degradation of EEP compounds present at higher concentrations. The second occurred within the same temperature range observed for the 5% and 10% EEP samples ($T_{\text{onset}} = 338^\circ\text{C}$; $T_{\text{max}} = 358^\circ\text{C}$) and was predominantly associated with the thermal degradation of the NR matrix, with possible contribution from EEP constituents present in the membrane [45, 46].

The DSC curves of NR (Fig. 3D–F) exhibited a single thermal event corresponding to its glass transition temperature (T_g) at -65°C , which is characteristic of the amorphous poly(cis-1,4-isoprene) structure [49, 50]. In contrast, the DSC curve of PDE (Fig. 3D) exhibited a pronounced endothermic peak at 207°C , corresponding to a melting enthalpy (ΔH) of 53.42 J/g . This thermal event is likely associated with the melting of phenolic compounds present in PDE [29, 49]. The incorporation of PDE into the NR matrix did not alter the T_g (-65°C), indicating that the chemical structure of NR, as well as the mobility of its polymer chains and the flexibility of the membrane, were preserved [51]. However, the NR + PDE 5%, NR + PDE 10%, and NR + PDE 20% samples exhibited an endothermic event at 192°C ($\Delta H = 1.67\text{ J/g}$), 182°C ($\Delta H = 0.78\text{ J/g}$), and 166°C ($\Delta H = 0.56\text{ J/g}$), respectively. These events were attributed to the melting of phenolic compounds present in PDE.

AEP (Fig. 3E) exhibited a single pronounced endothermic event at approximately 157°C ($\Delta H = 1535.90\text{ J/g}$), corresponding to the melting of the compounds present in AEP [52, 53]. The incorporation of 5%, 10%, and 20% AEP into NR resulted in a shift in the T_g (-65°C), accompanied by a reduction in its intensity. Specifically, NR + AEP 5% and NR + AEP 10% exhibited a T_g of -3°C , while NR + AEP 20% showed a T_g of -6°C . This shift toward higher temperatures indicates reduced polymer chain mobility, reflecting increased material rigidity after AEP incorporation, suggesting a reduction in elasticity compared to NR without propolis [54, 55]. Additionally, a shift was observed in the endothermic event associated with the melting of AEP compounds (157°C). The NR + AEP 5%, 10%, and 20% samples exhibited melting temperatures (T_m) of 181°C ($\Delta H = 183.59\text{ J/g}$), 179°C ($\Delta H = 181.78\text{ J/g}$), and 171°C ($\Delta H = 172.90\text{ J/g}$), respectively. This increase in T_m suggests a protective effect

of the NR matrix on the AEP compounds, providing enhanced thermal stability to the incorporated bioactive components [56].

As observed for PDE (Fig. 3D) and AEP (Fig. 3E), the DSC curve of EEP (Fig. 3F) also exhibited a single pronounced endothermic event near 135 °C ($\Delta H = 840.76$ J/g), attributed to the melting of its organic constituents [55]. The incorporation of different concentrations of EEP into the NR matrix did not promote any shift in the T_g (-65 °C), indicating that the mobility of the polymer chains in the amorphous phase was preserved. However, a reduction in the intensity of the event associated with T_g was observed, suggesting a possible partial restriction of segmental relaxation due to intermolecular interactions between the extract components (EEP) and the elastomeric matrix (NR). Additionally, all NR + EEP formulations (5%, 10%, and 20%) exhibited an endothermic event near 0 °C (highlighted in the graph), which is likely associated with the presence of residual solvent, such as water or ethanol, consequently resulting in a baseline shift [57]. Further endothermic events were observed in the EEP-containing samples, varying according to extract concentration. Specifically, NR + EEP 5% presented an event at 150 °C ($\Delta H = 148.30$ J/g), NR + EEP 10% exhibited two events at 145 °C and 184 °C ($\Delta H = 4.42$ J/g and 177 J/g, respectively), and NR + EEP 20% showed an event at 135 °C ($\Delta H = 16.49$ J/g). These events can be attributed to thermal transitions and/or melting processes of different organic fractions present in the EEP, whose contribution becomes more evident as their concentration in the polymer matrix increases.

Integrating the DSC and TGA results, it is evident that the incorporation of propolis extracts (PDE, AEP, and EEP) does not compromise the thermal stability of the NR matrix, whose main degradation event remained unchanged (~360 °C). The maintenance of the T_g (-65 °C) in formulations containing PDE and EEP confirms that the mobility of the amorphous NR phase was preserved, whereas the shift in T_g observed in the AEP-containing samples indicates increased segmental restriction and enhanced rigidity of the system. The additional endothermic events observed between ~135 °C and 192 °C are associated with thermal transitions and/or the melting of the phenolic fractions of propolis, in agreement with the degradation stages identified by TGA. Furthermore, the ΔH values associated with the thermal events of PDE and AEP decreased with increasing extract concentration, reflecting greater dispersion in the amorphous NR matrix and corroborating the SEM analysis (Fig. 2). Overall, these results indicate that NR acts as a thermally stable and partially protective matrix, preserving its structural integrity while effectively incorporating the bioactive constituents of propolis.

Contact angle and swelling properties assessment

Contact angle measurements were performed to assess the wettability of NR membranes after the incorporation of 5%, 10%, and 20% of different types of propolis (PDE, AEP, and EEP). As shown in Fig. 4A-C, although all samples exhibited statistically significant differences within each group (** $p < 0.001$), the contact angles remained below 90° for all formulations analyzed (NR, NR + PDE, NR + AEP, and NR + EEP). Contact angles below 90° are indicative of increased hydrophilicity, whereas angles above 90° are associated with hydrophobic surfaces [58, 59]. The

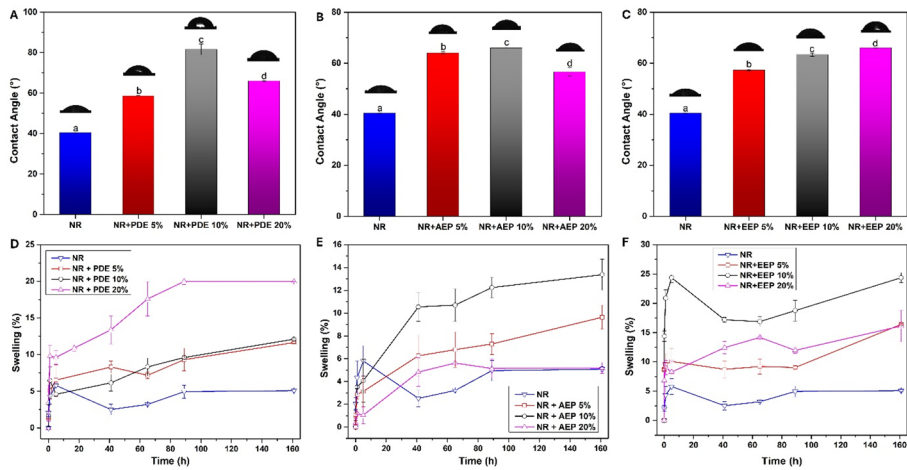


Fig. 4 Contact angle and the typical appearance of water droplets on the surface of natural rubber (NR) membranes and NR incorporated with different concentrations (5%, 10%, and 20%) of (A) propolis dry extract (PDE), (B) aqueous extract of propolis (AEP), and (C) ethanolic extract of propolis (EEP). Different letters indicate statistically significant differences (***) $p < 0.001$). Degree of swelling membranes of (D) NR+PDE (E) NR+AEP, and (F) NR+EEP. Statistical significance between groups is * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

sample composed solely of NR exhibited the lowest contact angle measured ($40.51^\circ \pm 0.08$) compared to the other formulations. A similar result was observed by Silva et al. [20], in which the NR membrane exhibited a reduced contact angle ($44.4^\circ \pm 3.7$). Although NR films typically exhibit relatively high contact angles, associated with the predominantly hydrophobic nature of the isoprene moieties [60], the presence of residual proteins originating from NR may alter the surface properties of the material. The incomplete removal of these proteins during the deproteinization process results in the persistence of polar functional groups on the NR surface, which increase the affinity for water and enhance wettability, thereby justifying the observed reduction in the contact angle [6].

The incorporation of different types and concentrations of propolis (PDE, AEP, and EEP) into the NR matrix resulted in an overall increase in contact angles compared with the pure NR sample. However, this increase was not sufficient to alter the hydrophilic character of the membranes, as all samples exhibited contact angles below 90° and maintained their hydrophilic nature after propolis incorporation.

Accordingly, NR+PDE (Fig. 4A) presented contact angles of $58.72^\circ (\pm 0.04^\circ)$, $81.60^\circ (\pm 2.67^\circ)$, and $66.09^\circ (\pm 0.19^\circ)$ for 5%, 10%, and 20% PDE, respectively. Similarly, the NR+AEP formulations (Fig. 4B) exhibited contact angles of $64.08^\circ (\pm 0.45^\circ)$, $66.02^\circ (\pm 0.37^\circ)$, and $56.67^\circ (\pm 1.62^\circ)$ for 5%, 10%, and 20% AEP, respectively. In turn, the NR+EEP samples (Fig. 4C) showed a concentration-dependent increase in contact angle, reaching $57.32^\circ (\pm 0.32^\circ)$, $63.36^\circ (\pm 0.65^\circ)$, and $66.09^\circ (\pm 0.31^\circ)$ for 5%, 10%, and 20% EEP, respectively.

In turn, the observed increase in contact angles following the incorporation of propolis can be attributed to changes in the surface chemistry of the NR membrane. As observed by SEM (Fig. 2), the presence of propolis extracts, which may con-

tain a complex mixture of compounds, including waxes and fatty acids [61, 62], can introduce less polar or hydrophobic groups on the NR surface, altering the surface energy distribution and promoting greater initial resistance to water spreading. These findings are consistent with Ebrahimi et al. [63], who reported that the addition of propolis decreased the hydrophilicity of electrospun chitosan/polyethylene oxide membranes, confirming the hydrophobic nature of propolis. Furthermore, materials with moderate hydrophilicity can influence cell adhesion and proliferation while also providing a protective role in damaged tissue and maintaining tissue hydration [6].

The swelling capacity of the samples, including NR and NR incorporated with different concentrations (5%, 10%, and 20%) of propolis (PDE, AEP, and EEP), are shown in Fig. 4D–F. The NR membrane exhibited a swelling capacity of approximately 6% within the first 5 h, maintaining this behavior over a period of 161 h. Studies by Asami et al. [6] indicate that deproteinization of NR reduces its swelling capacity, as the removal of proteins and lipids from the colloidal surface exposes hydrophobic chains, thereby decreasing water absorption. Furthermore, the reduced swelling capacity observed for NR may be associated with its smooth surface and the absence of pores, as evidenced by SEM analysis (Fig. 2).

Both NR and the samples containing different concentrations (5%, 10%, and 20%) of NR+PDE (Fig. 4D) and NR+AEP (Fig. 4E) exhibited no significant differences in swelling behavior during the first 5 h of analysis. However, within the same time frame, the NR+EEP 10% sample (Fig. 4F) exhibited a swelling capacity of approximately 24%, showing a significant difference compared to NR (** $p < 0.001$) and the NR+EEP 5% and NR+EEP 20% samples (** $p < 0.01$), which did not differ statistically from each other.

As observed in Figs. 4D–F, the incorporation of different types of propolis resulted in an overall increase in the swelling capacity of NR membranes over the course of the analysis. Accordingly, the swelling profile of NR + PDE (Fig. 4D) showed a progressive increase in swelling capacity with the gradual rise in PDE concentration in NR. At the end of the analysis (161 h), both NR + PDE 5% and NR + PDE 10% did not exhibit significant differences in swelling ($\sim 12\%$). However, they showed an extremely significant difference (** $p < 0.001$) compared to NR and NR + PDE 20%. In contrast, NR + PDE 20% displayed a significantly higher swelling capacity (** $p < 0.001$) compared to the other samples, reaching an approximate 20% increase in volume. This effect can be attributed to the presence of carboxyl groups in the PDE composition, which can establish hydrogen bonds and engage in both inter- and intramolecular interactions with the hydroxyl groups of PDE and with water molecules. This mechanism enhances water absorption and retention, resulting in an increased swelling capacity of the samples [55, 64].

The samples containing AEP (Fig. 4E) exhibited a distinct swelling behavior compared to PDE (Fig. 4D). After 161 h, the NR + AEP 5% and NR + AEP 10% samples exhibited similar swelling profiles, with values of approximately 10% and 13%, respectively, showing differences of * $p < 0.05$ and ** $p < 0.01$ compared to NR. In contrast, the NR + AEP 20% sample did not show a significant difference relative to NR, displaying a swelling behavior like that of the pure membrane. This behavior can be attributed to the hydrophobic compounds present in AEP, given that propolis is a chemically complex resinous bee product composed of waxes and vari-

ous organic compounds. This interpretation is supported by SEM analysis (Fig. 2), which revealed a greater dispersion of resinous particles originating from the propolis extracts remaining after the drying process, with this effect being more pronounced in the sample containing the highest propolis concentration (NR + AEP 20%). The incorporation of these constituents into the polymeric matrix may result in a relative increase in surface hydrophobicity, thereby reducing water diffusion and absorption and contributing to the water barrier effect observed in the membranes [19, 65].

Finally, NR incorporated with EEP (Fig. 4F) exhibited a swelling behavior distinct from that observed for NR+PDE (Fig. 4D) and NR+AEP (Fig. 4E). At the end of the analysis, the NR+EEP 5% and NR+EEP 20% samples exhibited similar swelling values (~16%), both significantly higher (** $p < 0.01$) than that observed for pure NR. Analogous to the behavior observed for NR+AEP 10% (Fig. 4E), the NR+EEP 10% sample displayed the highest swelling capacity (~24%), being significantly greater (***) $p < 0.001$) compared to the NR membrane.

Overall, the incorporation of different types of propolis (PDE, AEP, and EEP) altered the swelling behavior of the NR membranes. In addition to the waxy and resinous compounds responsible for hydrophobicity [66], propolis also contain flavonoids and other phenolic compounds, which may contribute to an increased affinity for polar media or compounds [65], resulting in effects that may vary depending on the type and concentration of propolis. This combination of chemical groups directly influences the water absorption capacity. Such capacity is mainly associated with the presence of polar groups, such as hydroxyl and carboxyl groups, which can establish hydrogen bonds with water molecules through inter- and intramolecular interactions, thereby promoting water absorption and retention [65]. Furthermore, the dispersion of the extracts within the NR matrix and the surface morphology of the membranes, as evidenced by SEM (Fig. 2) and characterized by greater irregularity compared with pure NR, increase the contact area with water and facilitate solvent penetration, further contributing to the enhanced swelling behavior.

Although the incorporation of propolis resulted in an increase in the contact angle compared to pure NR, all formulations-maintained values below 90°, indicating an overall hydrophilic character. The increase in contact angle can be attributed to changes in surface chemistry arising from the presence of less polar components of the propolis extracts, such as waxes and fatty acids, which may partially reduce the surface energy and increase the initial resistance to water spreading. Nevertheless, the simultaneous presence of polar groups in the phenolic constituents allows interaction with the aqueous medium over time, which explains the maintenance of hydrophilicity and the observed increase in swelling capacity.

Thus, the results suggest that the water absorption behavior of the membranes does not depend exclusively on the initial surface wettability, but also on structural and chemical factors, including the presence of hydrophilic groups, the distribution of extract constituents, and the surface morphology. Taken together, these factors explain the increased swelling observed in propolis-containing membranes, even in the presence of moderately higher contact angles compared with pure NR.

Both hydrophobic and hydrophilic membranes exhibit potential for biomedical applications. Hydrophobic membranes act as protective barriers, preventing the infiltration of fluids and pathogens and allowing prolonged drug release through hydro-

phobic interactions. On the other hand, hydrophilic membranes, as observed for the NR + EEP samples, are particularly effective in exudate absorption and moisture retention, promoting tissue regeneration and wound healing [67]. This swelling capacity further reinforces the potential of these membranes as bioactive dressings, providing suitable conditions for the healing process [68–70].

In vitro release profile

The NR membranes incorporated with different concentrations (5%, 10%, and 20%) of PDE, AEP, and EEP exhibited a biexponential release profile (Fig. 5). This profile was characterized by an initial burst release phase during the first 5 h, mainly attributed to the diffusion of propolis molecules adsorbed on the sample surfaces. Following this initial stage, a second phase of slower and more gradual release was observed between 20 and 140 h, which was predominantly attributed to the diffusion of propolis molecules from the interior of the polymeric matrix into the external medium [38].

Statistical analysis revealed that the NR + PDE 5%, NR + PDE 10%, and NR + PDE 20% samples (Fig. 5A) did not exhibit statistically significant differences in their release profiles during the first 5 h of analysis. Both the NR + PDE 5% and NR + PDE 10% samples showed similar release profiles throughout the entire analysis period, reaching a total release of approximately 20.0 µg/mL after 140 h. In contrast, the NR + PDE 20% sample exhibited a statistically significant difference (** $p < 0.01$) compared to the other formulations, showing a higher release capacity, which can be attributed to the greater PDE concentration incorporated into the polymeric matrix. At the end of 140 h, this formulation reached a release of approximately 26.9 µg/mL of PDE.

In turn, the release of the compound from the NR + AEP samples (Fig. 5B) exhibited a concentration-dependent behavior, increasing with the amount of AEP incorporated into the polymeric matrix. Within the first 5 h, the NR + AEP 20% sample exhibited a significantly higher release profile compared to NR + AEP 5% (***) ($p < 0.001$) and NR + AEP 10% (** $p < 0.01$). At this point, the released concentrations were 21.2, 57.5, and 93.0 µg/mL for NR + AEP 5%, NR + AEP 10%, and NR + AEP 20%, respectively. This significant difference persisted until 140 h, resulting in final released concentrations of 34.5, 112.2, and 204.3 µg/mL for the respective samples. The progressive release indicates a diffusion of active compounds is modulated by the AEP content, which may be advantageous for applications requiring prolonged and controlled release. Furthermore, the higher release profile observed for the NR sample containing the highest AEP concentration (20%) is consistent with the SEM analyses (Fig. 2), which revealed a greater presence of resinous fractions on the surface of this sample.

The release of EEP (Fig. 5C) initially appeared to be concentration-dependent, associated with a high release capacity. The NR + EEP 5% membrane showed an initial release of 51.9 µg/mL, reaching 133.5 µg/mL after 140 h. Similarly, the NR + EEP 10% and NR + EEP 20% formulations exhibited comparable release profiles, reaching 83.2 µg/mL and 91.9 µg/mL at 5 h, respectively, and attaining final values of 216.7 µg/mL and 253.0 µg/mL. However, statistical analysis revealed no significant differences in the release profiles among the formulations at either the initial (5 h)

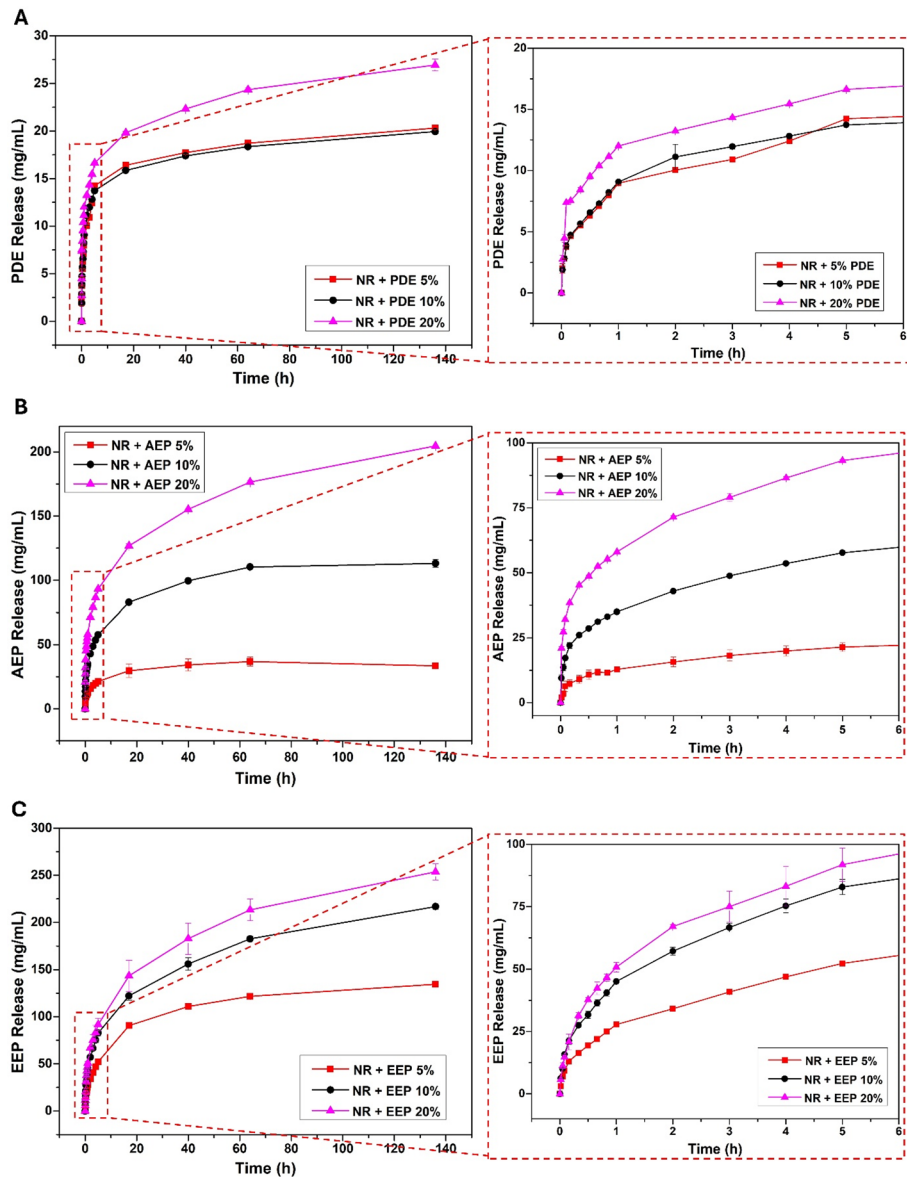


Fig. 5 Propolis in vitro release profile. The graph shows the release profile of **(A)** propolis dry extract (PDE), **(B)** aqueous extract of propolis (AEP), and **(C)** ethanolic extract of propolis (EEP) from natural rubber (NR) membranes with different concentrations of PDE (5%, 10%, and 20%). Statistical significance between groups is * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

or final (140 h) time points. This finding is consistent with the swelling analysis (Fig. 4F), which showed only minor differences among the propolis-containing membranes, suggesting that structural and morphological features of the NR matrix have a greater influence on both swelling and compound release than the EEP concentration.

These results indicate that the incorporation of propolis into the NR matrix directly influences the release profile, demonstrating a controlled and sustained system over time. Initially, the samples exhibited a burst release during the first 5 h of analysis, a behavior associated with the release of propolis adsorbed on the surface of the polymeric matrix, regardless of the type used, as evidenced by SEM analysis (Fig. 2). After this period, the release proceeded more gradually, reaching a stable plateau [38].

This profile suggests that the NR polymeric matrix functions as an efficient reservoir, modulating the release of propolis over time. The initial rapid release phase may be beneficial for providing an immediate effect, while the sustained phase contributes to prolonged action, which can be advantageous for biomedical applications, such as controlled-release systems in bioactive dressings or therapeutic devices [71, 72]. Moreover, the observed release kinetics emphasize the importance of the interaction between the polymeric matrix and the active compounds of propolis, highlighting the potential of this material for prolonged bioactive release applications.

Study release mechanism

To understand the mechanism associated with the release of the different types of propolis (PDE, AEP, and EEP) in the NR membrane, the experimental data obtained from the release profiles (Fig. 5) were fitted to the Korsmeyer-Peppas, Higuchi, First-order, and Zero-order kinetic models. The parameters obtained from these fittings, related to the release of PDE, AEP, and EEP, are presented in Tables 2 and 3, and 4, respectively.

The release behavior of the different types of propolis (PDE - Fig. 5A, AEP - Fig. 5B, and EEP - Fig. 5C) incorporated into the NR membrane was best described by the Korsmeyer-Peppas kinetic model, which showed the best fit to the experimental data compared to other models analyzed. Tables 2 and 3, and 4 demonstrate that the kinetic model applied to all samples was satisfactory, as the R^2 values were equal to or greater than 0.95, indicating good performance in describing the in vitro release profile.

This model is used to describe the release profile of the substance when the pre-dominant mechanism involves both Fickian diffusion and Type II transport (non-

Table 2 Kinetic models. Values of Korsmeyer-Peppas, Higuchi, First-order, and Zero-order mathematical models adjusted to the release profile of PDE

Kinetics models	Parameters	5% PDE	10% PDE	20% PDE
Korsmeyer-Peppas	R^2	0.95	0.93	0.96
	n	0.26	0.26	0.20
Higuchi	R^2	0.74	0.71	0.77
	b	0.38	0.21	0.16
	k	0.11	0.06	0.04
First-order	R^2	0.28	0.26	0.31
	$\ln Q_\infty$	0.83	0.85	1.18
	k	0.01	0.01	0.01
Zero-order	R^2	0.47	0.45	0.53
	b	0.52	0.27	0.21
	k_0	0.008	0.004	0.003

Table 3 Kinetic models. Values of Korsmeyer-Peppas, Higuchi, First-order, and Zero-order mathematical models adjusted to the release profile of AEP

Kinetics models	Parameters	5% AEP	10% AEP	20% AEP
Korsmeyer-Peppas	R^2	0.99	0.99	0.99
	n	0.28	0.29	0.26
Higuchi	R^2	0.87	0.92	0.91
	b	0.38	0.36	0.49
	k	0.25	0.26	0.23
First-order	R^2	0.34	0.46	0.51
	$\ln Q_\infty$	1.17	2.25	2.80
	k	0.03	0.03	0.03
Zero-order	R^2	0.65	0.73	0.73
	b	0.61	0.59	0.67
	k_0	0.029	0.031	0.032

Table 4 Kinetic models. Values of Korsmeyer-Peppas, Higuchi, First-order, and Zero-order mathematical models adjusted to the release profile of EEP

Kinetics models	Parameters	5% EEP	10% EEP	20% EEP
Korsmeyer-Peppas	R^2	0.98	0.98	0.98
	n	0.41	0.39	0.41
Higuchi	R^2	0.96	0.94	0.94
	b	0.74	0.65	0.55
	k	1.37	0.60	0.56
First-order	R^2	0.42	0.78	0.39
	$\ln Q_\infty$	1.84	1.11	2.42
	k	0.04	0.08	0.03
Zero-order	R^2	0.68	0.41	0.75
	b	2.33	2.33	1.16
	k_0	0.088	0.036	0.046

Fickian), the latter being influenced by the relaxation of polymer chains. When the release of the compound is primarily controlled by the diffusional process, the exponent value n is less than 0.5 ($n < 0.5$). On the other hand, when n equals 1 ($n = 1$), the diffusion process is characterized as Type II Transport, where the diffusion rate exerts greater influence on the release of the substance compared to the relaxation of the polymer chains [73].

The values of $n < 0.5$ observed for all samples indicate that the release profile in the system follows a Fickian diffusion model, suggesting that diffusion is the main mechanism responsible. This type of diffusion is commonly observed in dilute solutions [74]. This behavior explains the relatively slower release of propolis as the diffusion distance increases. Thus, the initial rapid release of propolis in the first hours can be attributed to the proximity of the substance to the membrane surface, resulting in a shorter distance to be traversed to the surface of the NR membranes [75].

Conclusion

FTIR spectra confirmed the successful incorporation of propolis extracts (PDE, AEP, and EEP) into the NR matrix, indicating physical interaction between the components that may facilitate the release of bioactive compounds, as observed in the

in vitro release profile. The addition of propolis favored controlled and sustained release, with the Korsmeyer-Peppas kinetic model providing the best fit ($R^2 > 0.95$). SEM analysis revealed that pure NR membrane exhibited a smooth and homogeneous surface, whereas propolis incorporation (5–20%) increased surface roughness and the presence of resinous particles, particularly at higher concentrations. TGA results suggested that propolis did not significantly alter the thermal behavior of NR, which acted as a protective matrix slowing thermal decomposition of propolis. DSC analysis showed that AEP and EEP incorporation did not affect the glass transition temperature (T_g) of the polymer matrix but generated endothermic events, possibly related to the melting transition of the propolis constituents. Propolis incorporation slightly increased the contact angle, yet all formulations remained below 90° , indicating an overall hydrophilic character. NR membranes containing EEP exhibited higher swelling capacity, reflecting the hydrophilic character. This fluid absorption is relevant for wound healing, as it helps maintain a moist environment that supports tissue regeneration. The combination of controlled bioactive release, swelling capacity, and exudate absorption creates a favorable environment for healing. Although biological assays were not performed, the physicochemical data suggest that these membranes may offer an innovative, low-cost therapeutic alternative.

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Data availability Data would be available on request.

Declarations

Competing interests The authors declare no competing interests.

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Authors and Affiliations

Evelin Thayná Barbosa Serpa^{1,2}  · Bruna V. Quevedo^{2,3}  ·
Eliana Aparecida de Rezende Duek^{2,3}  · Maira de Lourdes Rezende^{1,2} 

✉ Bruna V. Quevedo
brunaquevedo@estudante.ufscar.br

¹ Department of Polymer, José Crespo Gonzales Faculty of Technology (FATEC), Sorocaba 18013-280, SP, Brazil

² Postgraduate Program in Materials Science (PPGCM), Federal University of São Carlos (UFSCar), Sorocaba 13565-905, SP, Brazil

³ Laboratory of Biomaterials, Faculty of Medical Sciences and Health (FCMS), Pontifical Catholic University of São Paulo (PUC-SP), Sorocaba 18030-070, SP, Brazil